Clinical Heterogeneity in Epidermolysis Bullosa: Speculations on Causation and Consequence for Research

A recent editorial in this journal presented an extensive review on the role of anchoring fibril changes in dystrophic epidermolysis bullosa (EBD), summarizing electron microscopic (EM), biochemical, and other evidence [1]. Included were highly relevant points on clinical diagnosis, not the least of which was the difficulty in discriminating the local forms of the recessive type (R-EBD) from sporadic dominant mutant (D-EBD) cases. The focus here will be on the clinical variations in different categories of EB, their possible causations, and consequences.

The clinical appearance of EB seems to have no end in its variation. Without knowing the cause for this, we may be unable to reach a sufficiently precise diagnosis to predict the fate of the individual patient. How much misleading advice has been given parents of the blistering infant? We are not far removed from the days when parents of a child with severely blistering dominant mutant case of the herpetiform Dowling-Meara variety (D-EBH-DM) were told the child was going to die and the recurrence risk for additional children was 25%. Today, by all available criteria a generalized R-EBD case is expected to develop synechiae and mitten hands, yet some cases fail to do so. Sometimes this is ascribed to the effect of phenytoin therapy, yet it occurs in the absence of such therapy! Is this because we do not yet recognize the inverse-dystrophic (R-EBD-I) type in infancy, or because of extensive trauma protection in the first months of the child, or because of a peculiar genetic makeup of the individual as, for example, different mutations in the paternally and maternally derived EB genes? Most surprises, however, may be experienced in the growing numbers of patients misdiagnosed in the past as having EBD, yet now by EM or by immunofluorescence shown to have junctional blistering with (or without?) hemidesmosome abnormalities.

Our goal today should be no less than to pinpoint the responsible mutated gene, pick it out of the DNA from the individual patient, cut it by restriction enzymes, and DNA-sequence it. The demonstrated change on the molecular level can then be correlated to other biochemical, morphologic, and clinical observations. To do so we must know precisely either where the gene is or what it does. At present we do not know either of these facts for any EB gene. Nor do we know how many *genetic disease entities* (how many different gene loci) are involved, nor how many types of mutations in and around each gene locus (allelic mutations) give different phenotypic results (*disease variants*).

Twenty-five years ago it was written in textbooks that EB was the best-defined genetic disease group in dermatology. At that time it comprised 3 different diseases. A few years ago we considered a minimum of 9 entities (gene loci) because EM had pinpointed primary or early changes of 9 different kinds, and 16 different clinicogenetic types when clinical signs, predilection sites, and course of disease were considered [2]. However, the variations and clinical complexity do not cease. What are the causes for this, and how does this influence research in EB?

INTRAEPIDERMAL EPIDERMOLYSIS BULLOSA

The typical EB simplex (D-EBS) patient presenting in warm weather with blistering soles, toes, and sometimes palms and fingers, may tell about having had occasional blisters elsewhere in childhood (mild generalized Köbner type, EBS-K), or deny this (localized Weber-Cockayne type, EBS-WC), and only rarely describe persistent generalized blister liability (severe Köbner type, EBS-K). Low intra- and high interfamilial variations indicate different mutations in different families [3]. There is as yet no proof that these occur in more than one gene locus, and linkage studies have only suggested this locus (*EBS2*) is on chromosome 1 [4]. However, the pooled EBS-K plus EBS-WC mutation rate of 2 in one million gametes per generation is 4 times higher than for any other dominant EB-type [2] and gonadal mosaicism for new mutations is relatively frequent as disclosed by 2 or more patients in the first affected generation (Anton-Lamprecht, Gedde-Dahl, Ledaal, unpublished observations) [3]. The *EBS2* locus must fall into a very special genetic category, or genetic heterogeneity exists. Low expression of fibroblast gelatinase activity has been reported in 3 of 9 D-EBS-WC cases [5], 3 of 9 D-EBS-K cases* [5], but in none of 40 non-EBS persons* [5]. The significance of this is unclear. Possible clinical hints of heterogeneity can be that some, but not all, families show dramatic improvement with age; some, but not all, report their serous blisters frequently turn hemorrhagic; and rare cases display palmoplantar erythema and initial blistering in palm before foot blistering.*

Multiple erosions on the hands should alert the clinician to ask about epidermal fragility since this is typical for a distinct entity, D-EBS-Ogna [3], caused by mutation at the *EBS1* locus which is linked to the glutamic pyruvic transaminase locus (*GPT*) on chromosome 8 or 16 [2].

The adolescent or adult patient who may clinically be suspected of having EBS, but who fails to exacerbate in warm weather and who had severe blistering in infancy and preschool years when fever cleared the skin, will in all probability by EM show basal cell subnuclear tonofibrillar aggregation in perilesional skin biopsies. Epidermolysis bullosa herpetiformis Dowling-Meara (D-EBH-DM) is definitely a disease separate from the basal cell cytolysis EBS [2]. In infancy it is confused with junctional EB and in childhood with dystrophic EB.

Lastly, Bart's type of EB seems to fall in the intraepidermal group [2], but EM needs to be performed on the original family for verification.

JUNCTIONAL EPIDERMOLYSIS BULLOSA

Following Herlitz's description in 1935 of EB letalis as nonscarring and lethal within 3 months, and Pearson's discovery in 1962 that blisters formed in the intermembrane (junctional) space between the basal cell plasma membrane and the electron-dense dermal membrane, junctional EB (EBJ) was for a long time considered to be synonymous with Herlitz's disease with occasional survivors. The abnormality of hemidesmosomes, first recognized by Hashimoto et al in 1976 [2], then allowed identification of EBJ in 2 ways: either by a junctional split or, even in nonblistered skin, by lack of a subbasal dense plate and often other abnormalities of the hemidesmosomes. However, this was soon found in patients clinically separate from the Herlitz type. A significant point was that several of the non-Herlitz EBJ patients were of consanguineous parentage, indicating both parents carried the

*Winberg, Gedde-Dahl: Gelatinase expression in generalized epidermolysis bullosa simplex fibroblasts. Submitted for publication.

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same mutant gene. This was found for an adult with blistering legs only, for 4 of 5 Norwegian families by 1980 singled out to have the junctional form of EB inversa (R-EB atrophicans inversa [2] or R-EBJ-I), and for the Austrian kindred with *atrophic benign* EBJ [6] (R-EB atrophicans mitis [2] or R-EBJ-M). These findings support at least 3 different types of EBJ-mutations: a Herlitz mutation, an inversa mutation, and a mitis mutation.

The prototype homozygotes for each of these recessive genes are well known and differ clinically in several respects. Herlitz alone develops the perioral-perinasal crusted lesions from age of 3–5 months; inversa alone has total normalization of skin after 2–3 months of age and the later reappearance of blisters with preference for the groins and axillae; mitis has persistent generalized blisters on both the extremities and the trunk. Signs in common but prevailing in inversa are the recurrent corneal erosions—particularly during the otherwise oligosymptomatic period at 6 months to 3 years of age; prevailing in Herlitz and lacking in inversa is the recurrent laryngeal involvement with hoarseness. Signs in common are the pronounced enamel defect and the peculiar type of nail changes. The large nonhealing lesions on the buttocks, and often also elsewhere, characterize the end stage in both the Herlitz and inversa types, but with a 50- to 70-year difference in life span. The epidemiologies of the 3 mutant genes are also different, the Herlitz gene being for example very common in Sweden [2], Germany [7], and presumably in England [8], but rare in Norway [2] and Finland [9].

Turning then to EBJ without known parental consanguinity or evidence favoring common origin of parental genes, the clinical variation seems increasingly manifold. First, for the long-term Herlitz survivors (defined by persistent facial eruption), the severity of disease is strikingly different among families [2] and the 2 cases made accessible to me have both had ancestries strongly arguing against inbreeding. Can there be more than one Herlitz mutation? What happens when one of each meet in a genetic compound patient? Second, 2 adult EM-verified EBJ patients in Sweden (Anton-Lamprecht, Gedde-Dahl, Ledaal, unpublished observations) differed both in their generalized childhood and in benign adulthood blistering from all EBJ types mentioned above, still both are very unlikely of consanguineous parentage. Combining this with the very high Herlitz-gene frequency in Sweden, and the absence of similar EBJ patients in Norway, one may wonder whether there could be 2 kinds of Herlitz genes in Sweden, genes which could partly complement one another's defect in the noninbred genetic compound patient? Third, we are presently observing 2 Norwegian dizygotic twin brothers with a Herlitz-like evolution of the disease at age 6 months (Anton-Lamprecht, Gedde-Dahl, Ledaal, unpublished observations) yet their father is by ancestry a very probable carrier of the inversa gene. They are therefore candidates for being Herlitz/inversa compounds.

The hypothesis of allelism for several distinct EBJ mutations is attractive and justifies a search for the gene locus (*EBR2*) pooling all types of EBJ families with hemidesmosome abnormalities. Furthermore, in the present state of outbreeding, particularly on the American continent, the prediction of a baby's prognosis from EM verification EBJ is indeed difficult. A recent morphometric EM study did not reveal distinct differences in the hemidesmosome abnormality in different clinical variants of EBJ [8] in conformity with the experience of others (Anton-Lamprecht, Gedde-Dahl, Ledaal, unpublished observations). A more serious question raised by Tidman and Eady's [8] study is the diagnosis of EBJ in individuals found to have apparently normal hemidesmosomes. More documentation is needed on this point.

Junctional blistering with normal hemidesmosomes is found in recessive EB progressiva, a juvenile-onset disease never clinically confused with the hemidesmosome EBJ diseases, but with localized EBS in adolescence and acquired EBD later on [2]. The slowly progressive distal skin atrophy with loss of digital dermal ridges and lingual papillae pinpoint R-EBP as almost certainly a distinct entity. Until recently a "European" disease, it is now also recognized in Canada (R. M. Haber, personal communication). Its gene locus (*EBR3*) is not chromosomally mapped.

DERMOLYTIC EPIDERMOLYSIS BULLOSA

Blister formation beneath the dermal membrane leads to red atrophic, sharply demarcated scarring after repeated blisters. Dermolytic EB is now widely used synonymously with EBD, ranking from D-EBD and R-EBD to acquired types. The role of anchoring fibrils (AF) and collagenase abnormalities have been thoroughly reviewed [1]. The D-EBD families in which the patients develop white (albopapuloid) papules on the trunk usually first at adolescence (D-EBD-Pasini), have an early onset (neonatally) and more extensive blistering and scarring on the extremities than do the majority of non-Pasini or Cockayne-Touraine (D-EBD-CT) families. This picture is complicated by the facts that Pasini families can include patients who do not develop the papules and that occasional D-EBD families do not show the papules despite blistering and scarring closely resembling D-EBD-P (Anton-Lamprecht, Gedde-Dahl, Ledaal, unpublished observations). Dominant dystrophic epidermolysis bullosa with generalized blistering is quite exceptional, also in lacking Pasini papules [10], but all D-EBD patients seem to lack any tendency to pseudosyndactyly. In this case pseudodominant recessive inheritance must critically be evaluated. That D-EBD-P has generalized and D-EBD-CT localized AF abnormality [2] has not been confirmed by a recent morphometric study [10], although biopsy sites were not the same. Linkage studies in progress may soon solve the problem of genetic heterogeneity.

The discrimination between localized R-EBD and a D-EBD mutant is an extremely important but difficult task for genetic counseling. Visible changes in collagen fibrils suggesting excess collagenolysis are found only in R-EBD by some investigators [2] and this discriminator was not evaluated by the morphometric study, showing that AF changes were useless for the same purpose [10]. Anti-AF antibodies react poorly or not at all with R-EBD-skin [11,12]. Another recent monoclonal antibody showed continuous staining of the sublaminar AF zone in normal and D-EBD skin, patchy staining in local R-EBD skin, and no or markedly reduced staining in generalized R-EBD skin [13]. From pedigree evidence [3], there is good reason to assume that local and generalized (mutilans) R-EBD is due to different mutations in the same gene locus, and that the genetic compound carrying one of each gene will be clinically intermediate in severity. The monoclonal antibodies, together with the autoantibodies revealing the new basal lamina "acquired epidermolysis bullosa antigen," may hold promise in the search for the D-EBD and/or R-EBD gene products [12] as well as for the genes themselves. The relationship between the collagenase locus on chromosome 11 [14] and the *EBR1* locus for R-EBD is not yet clear [1].

The inverse-dystrophic (R-EBD-I) type, despite being indistinguishable from the common R-EBD (R-EBD-HS) type by EM, has such a distinctive expression of scarring blistering on the trunk while sparing the extremities, that a separate genetic entity must be assumed [2,9]. Follow-up of one of our patients who has developed repeated keratoacanthomas, leads us to suspect that some of the ancient reports on "ulcero-vegetant" EB may have been R-EBD-I, although others may have been R-EBJ.

We are in the stage of having been delivered most pieces of a 1000-piece jigsaw puzzle of clinical observations. How fast can we put them together correctly? The patients want to know. Their families want to know. To do meaningful research, we must try, by trial and error, over and over again.

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